

Human RNA Polymerase II Subunit hsRPB7 Functions in Yeast and Influences Stress Survival and Cell Morphology

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Using a screen to identify human genes that promote pseudohyphal conversion in *Saccharomyces cerevisiae*, we obtained a cDNA encoding hsRPB7, a human homologue of the seventh largest subunit of yeast RNA polymerase II (RPB7). Overexpression of yeast RPB7 in a comparable strain background caused more pronounced cell elongation than overexpression of hsRPB7. hsRPB7 sequence and function are strongly conserved with its yeast counterpart because its expression can rescue deletion of the essential *RPB7* gene at moderate temperatures. Further, immuno-precipitation of RNA polymerase II from yeast cells containing hsRPB7 revealed that the hsRPB7 assembles the complete set of 11 other yeast subunits. However, at temperature extremes and during maintenance at stationary phase, hsRPB7-containing yeast cells lose viability rapidly, stress-sensitive phenotypes reminiscent of those associated with deletion of the *RPB4* subunit with which RPB7 normally complexes. Two-hybrid analysis revealed that although hsRPB7 and *RPB4* interact, the association is of lower affinity than the *RPB4*-*RPB7* interaction, providing a probable mechanism for the failure of hsRPB7 to fully function in yeast cells at high and low temperatures. Finally, surprisingly, *hsRPB7* RNA in human cells is expressed in a tissue-specific pattern that differs from that of the RNA polymerase II largest subunit, implying a potential regulatory role for hsRPB7. Taken together, these results suggest that some RPB7 functions may be analogous to those possessed by the stress-specific prokaryotic sigma factor *rpoS*.

INTRODUCTION

Transcription of messenger RNAs in eukaryotes must be responsive to a large number of complex intracellular and environmental signals that affect many cell processes, including control of cell division, specification of morphology, and response to stress. To begin to understand how signals selectively influence the expression of genes involved in fundamental cell processes, much work has been directed toward understanding how transcription factors orchestrate the actions of basal transcrip-

tional machinery (reviewed in Buratowski, 1994; reviewed in Tjian and Maniatis, 1994). In addition to transcriptional regulation mediated through sequence-specific transcription factors and TATA-binding protein (TBP)-associated factors (TAFs), some evidence suggests that RNA polymerase II (pol II) may also influence gene expression by altering its subunit composition in response to nutrient and thermal stress (Edwards *et al.*, 1991; Choder, 1993; Choder and Young, 1993). Although prokaryotes typically modulate transcription in this fashion, using dissociating σ subunits to direct promoter specificity (Chamberlin, 1975), no single eukaryotic σ -like subunit has been identified.

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The subunit architecture and composition of RNA polymerases is well conserved in eukaryotes. The genes encoding all 12 *Saccharomyces cerevisiae* pol II subunits, designated RPB1-RPB12, have been cloned and characterized (Young, 1991; Treich *et al.*, 1992; Woychik *et al.*, 1993). A number of lines of evidence indicate that two of the subunits, RPB4 and RPB7, may play a σ -like role that is specifically required for stress response. First, in actively growing yeast cells, approximately 20% of pol II is purified as a species containing all 12 subunits, whereas 80% lacks the stable RPB4-RPB7 subcomplex (Edwards *et al.*, 1991). In yeast that are at stationary phase, a marked shift in the assembly of the RPB4-RPB7 subcomplex with pol II occurs, resulting in virtually 100% assembly of the subcomplex (Choder and Young, 1993). Second, yeast strains with null alleles for RPB4 (*rpb4*– strains) are inviable at low and high temperatures, and lose viability much more rapidly than wild-type yeast when kept at stationary phase (Woychik and Young, 1989; Choder and Young, 1993). This is attributable to transcription defects, as mRNA synthesis in *rpb4*– yeast declines rapidly after heat shock or during shift to stationary phase growth relative to RPB4⁺ yeast (Choder and Young, 1993). Third, yeast overexpressing RPB4 grow more rapidly than wild-type yeast during post-logarithmic growth phases (Choder, 1993). In sum, these results suggest that the RPB4/RPB7 complex increases the resistance of RNA polymerase II to stress, and thus contributes to organismal survival under inclement growth conditions.

We report here the isolation of a human RPB7 homologue *hsRPB7* through a genetic screen in *S. cerevisiae* similar to that recently described by Gimeno and Fink (1994). Overexpression of *hsRPB7* and, more pronouncedly, yeast RPB7 leads to enhanced production of pseudohyphae during growth on nitrogen-limited media. *hsRPB7* complements *rpb7*– yeast strains at moderate temperatures. However, cells growing under control of *hsRPB7* rapidly lose viability after culture at temperature extremes, and during maintenance at stationary phase. *hsRPB7* assembles with the complete set of yeast pol II subunits and directly interacts with RPB4, although with lower affinity than the RPB7-RPB4 interaction in wild-type yeast cells. *hsRPB7* is expressed in a highly tissue-specific manner in human cells, in a pattern that differs from that of the largest subunit of pol II. Cumulatively, our data indicate that an essential RPB7 function is conserved from yeast through humans, and that the subunit may selectively associate with pol II to regulate its function.

MATERIALS AND METHODS

Bacteria and Yeast Strains

The *Escherichia coli* strain DH5 α F' [F'/endA1 *hsdR17*(*r_K*–*m_K*+) *supE44* *thi-1* *recA1* *gyrA* (Nal^r) *relA1* Δ (*lacZYA-argF*) U169 (ϕ 80*lac* Δ (*lacZ*)

M15)] was used as a host for all cloning constructions. The *S. cerevisiae* strain CGx74 [MAT *a/atrp1::his6/trp1::his6*], similar to CGx31 (Gimeno *et al.*, 1992) and congenic with the S1278b genetic background (Grenson *et al.*, 1966), was used in the library screen in which *hsRPB7* was isolated. A related strain, CGx75 MAT *a/atrp1::his6/trp1::his6 ura3/ura3*, was used for study of RPB4-RPB7 interactions. The yeast strain WY-7 [MAT *a/a ura3–52/ura3–52 his3 Δ 200/his3 Δ 200 leu2–3,112/leu2–3,112 lys2 Δ 201/lys2 Δ 201 ade2/ade2 RPB7/*rpb7 Δ 1::LEU2*] (McKune *et al.*, 1993) and its haploid derivative WY-73 [MAT *a ura3–52 his3 Δ 200 leu2–3,112 lys2 Δ 201 ade2 rpb7 Δ 1::LEU2* [pRP729] were used in complementation studies. WY-76, WY-77, and WY-78 were derivatives of WY-73 that contain an additional plasmid pRS413, pRP730, or pRP731, respectively. WY-74 [MAT *a ura3–52 his3 Δ 200 leu2–3,112 lys2 Δ 201 ade2 rpb7 Δ 1::LEU2* [pRP730] and WY-75 [MAT *a ura3–52 his3 Δ 200 leu2–3,112 lys2 Δ 201 ade2 rpb7 Δ 1::LEU2* [pRP731] were obtained after 5-fluoroorotic acid selection against the *URA3* plasmid pRP729. The yeast strains EGY48 [MAT *a ura3 his3 trp13lexAop-leu2*] (Gyuris *et al.*, 1993) and EGY191 [MAT *a ura3 his3 trp13lexAop-leu2*] were used for immunoprecipitation studies and two hybrid assays. Yeast cells were grown either on YPD (rich) medium, or on complete minimal medium lacking combinations of amino acids to select for the presence of plasmids (Ausubel *et al.*, 1987–1994), except as noted below.*

Library Screen and Assessment of Pseudohyphal Growth

Oligo(dT)-primed cDNA prepared from mRNA from actively proliferating HeLa cells was cloned into the galactose-inducible yeast expression vector JG4–4 (2 μ , *TRP1*) (Gyuris and Brent, unpublished data). The resulting library was transformed into the yeast strain CGx74 by the procedure of Schiestl and Gietz (1989), and 3 \times 10⁶ primary transformants were obtained on *trp*– glucose plates. Colonies were scraped off the plates and pooled in a 50-ml Falcon tube. A solution of 65% glycerol, 10 mM Tris-HCl, pH 7.5, and 10 mM MgCl₂ in a volume equivalent to the packed cell volume was added, and the resulting slurry was stored in 1 ml aliquots at –70°C. For the screen, an aliquot was thawed, 250 μ l of slurry was diluted to OD₆₀₀ of 0.6 in YPD medium, and grown overnight at 30°C. The following morning the overnight culture had an OD₆₀₀ of 3.0; this culture was rediluted to an OD₆₀₀ of 0.15 into YP medium with 2% galactose and 1% raffinose, and grown for 5 h (approximately one doubling) at 30°C. Cells were pelleted at 1200 \times g for 5 min, washed twice with distilled water, and plated at a density of ~3000–5000 cells/10 cm plate of SLAHGR medium (6.7 g/l Difco Yeast Nitrogen Base without amino acids and ammonium sulfate [Difco Laboratories, Detroit, MI], 0.2 mM L-histidine hydrochloride, 0.05 mM ammonium sulfate, 20% Difco agar, 2% galactose, 1% raffinose), and grown at 30°C for 2–3 days. Plates were then scanned using a Wild dissecting microscope at 25 \times , and colonies with enhanced formation of pseudohyphal projections were picked to a master plate. Library DNA was isolated from these colonies (Hoffman and Winston, 1987), transformed into *E. coli*, and retransformed into fresh CGx74; cDNAs that again enhanced pseudohyphal formation were chosen for further analysis.

For subsequent assays of formation of pseudohyphae, CGx74 and CGx75 yeast were transformed with plasmids as described in the RESULTS section, plated to *trp*– or *ura*–*trp*– glucose to select transformants, then restreaked to SLAHGR plates. Pseudohyphae were photographed at 24–72 h after streaking.

Sequence Analysis

Both strands of the *hsRPB7* gene were sequenced using oligonucleotide primers to the JG4–4 vector and to internal *hsRPB7* sequence in combination with the Sequenase system (United States Biochemical, Cleveland, OH). Database searching was performed using the BLAST algorithm (Altschul *et al.*, 1990) and sequence analysis was

carried out using the package of programs from UWGCG (Dereux *et al.*, 1984).

Plasmid Constructions

To construct pEG202-hsRPB7, the original JG4-4-hsRPB7 clone was digested with *EcoRI* and *XhoI*, and cloned into the vector pEG202ATT (a derivative of pEG202 (Golemis *et al.*, 1994) modified so that the reading frame from *EcoRI* corresponds to ATT; Golemis, unpublished data) that had similarly been digested with *EcoRI* and *XhoI*. The resulting clone encoded a fusion protein in which the LexA coding sequences were followed by the sequence NSARG-GTLPAYLVWE followed by the hsRPB7 coding sequences.

To construct pEG202-RPB7, oligonucleotides EG34 (5'-GGG CAA TTG GCG TCG AGA ATG TTT TTT ATT AAA GAC CTT TC) and EG35 (5'-CCC CTC GAG ATG TTG CGG AGT AAC AAG TGA TTA AAT A) were used to amplify and add a 5' *MunI* site and a 3' *XhoI* site to ~540 bp containing the RPB7 coding sequence from the plasmid pRB718 (which contains a 2.8-kb genomic fragment encompassing RPB7 in the vector pGEM3Zf). This fragment was cloned into the *SmaI* site of pUC119, and the sequence was confirmed. The fragment was then excised using *MunI* and *XhoI*, and cloned into pEG202 cut with *EcoRI* and *XhoI*, to create a fusion protein in which the C-terminal end of LexA is followed by the amino acid sequence ELASR, followed by RPB7 coding sequences. To construct the plasmid JG4-4-RPB7, used for overexpression of the yeast subunit, a *MunI*-*XhoI* fragment from the pUC119/RPB7 plasmid was cloned into JG4-4 cut with *EcoRI* and *XhoI*.

Oligonucleotides EG36 (5'-GGG CAA TTG TCG GGA AAA ATG AAT GTT TCT ACA TCA ACC) and EG37 (5'-CCC CTC GAG ATA CAG TTA TTA ATA GAG TGT TTC TAG G) were used in a similar strategy to isolate and add *MunI* and *XhoI* sites to RPB4 coding sequences, using pRP415 (which contains a 2.3-genomic fragment encompassing RPB4 in the vector YEplac181 [Sikorski and Hieter, 1989]) as sequence donor. After confirmation of the correct sequence, the ~620 bp of coding sequence were excised from pUC119 using *MunI* and *XhoI*, and inserted into *EcoRI*- and *XhoI*-cut JG4-5, creating an in-frame fusion protein with the SV40 nuclear localization motif, the B42 transcriptional activation domain, and the HA1 epitope tag, for use in interaction analysis. The RPB4 coding sequence was similarly cloned from the pUC119 subclone into JG4-4 to express native RPB4 with a TRP1 selectable marker, or into pYES2 (Invitrogen, San Diego, CA) to express native RPB4 with a URA3 selectable marker.

The LexA-operator-LacZ reporter has been described (Golemis *et al.*, 1994). Briefly, it is a derivative of LR1Δ1 (West *et al.*, 1984) containing the 2 μm origin of replication and the URA3 selectable marker for propagation in yeast, and constructed so that four copies of LexA operator motifs derived from the *colE1* promoter (Ebina *et al.*, 1983) are located upstream of the GAL1 minimal promoter and the LacZ coding sequences.

The plasmid pRP729 has an ~1.4-kb RPB7-containing fragment with ~600 bp 5' *BamHI* ends and ~300 bp 3' *Sall* ends cloned into the *BamHI*/*Sall* sites of the URA3/CEN-ARS vector pRS416 (Sikorski and Hieter, 1989). pRP730 has the ~1.4 kb *BamHI*/*Sall* insert from pRP729 cloned into the *BamHI*/*Sall* sites of the HIS3/CEN-ARS plasmid pRS413 (Sikorski and Hieter, 1989). To construct plasmid pRP731, the RPB7 coding sequences of pRP730 were exactly replaced with those of *hsRPB7* using a two step polymerase chain method (PCR) method (Dillon and Rosen, 1990). First, two sets of oligonucleotides were used to create fragments representing the *S. cerevisiae* RPB7 5' sequences (oligo A, 5'-GCG GAT CCG CTC CTT TCC CTG CTC TAT GCC; oligo B, 5'-GCA GAT ATG GTA GAA CAT TCT CAG AAA TTG AG) and 3' sequences (oligo C, 5'-ACT TGG GGC TTG TAA GCT GAT CAC TTG TTA CTC CGC; oligo D, 5'-GCG TCG ACG AGG GGG AAT AGA TTC TTT AGC) using pRP729 as a template. These two PCR fragments contained ~15 bp of sequence (underlined regions of oligos B and C) complementary to the 5' and 3' ends of the *hsRPB7* gene. In the second PCR step,

three overlapping fragments (the two fragments created in the first step, along with the linearized JG4-4-hsRPB7 plasmid containing the *hsRPB7* coding sequence) were amplified using oligos A and D. The final PCR product containing the *hsRPB7* gene flanked by *S. cerevisiae* RPB7 promoter and 3' sequences was then transformed into WY-73 along with gapped pRP730 (which has the RPB7 gene and part of its 5' and 3' DNA removed after digestion with *SnaBI* and *MunI*) according to Muhlrad *et al.* (1992). Recombination between the complementary sequences in the PCR fragment and the vector occur during transformation, yielding plasmid pRP731 in the yeast strain WY-78.

Plasmid Shuffle

The yeast strains containing the yeast RPB7 HIS3 plasmid (WY-74) and the *hsRPB7* plasmid (WY-75) were obtained by selecting against the wild-type RPB7 URA3 plasmid using 5-fluoroorotic acid (Boeke *et al.*, 1987). This was achieved by transferring WY-77 and WY-78 cells to a his-leu- dropout plate containing 1 mg/ml of 5-fluoroorotic acid and selecting for growth at 30°C. As a control, the same procedure was done in parallel with the yeast strain containing the control HIS3 plasmid RS413 (WY-76) to demonstrate that this plasmid alone could not support yeast cell growth at 30°C.

To confirm that the correct plasmid was being expressed in *hsRPB7* cells, we rescued the plasmid from yeast cells and verified that it contained the *hsRPB7* gene (and not RPB7) using PCR with gene-specific oligonucleotides.

Growth Profiles

For studies of viability of yeast growing under control of *hsRPB7* versus RPB7, WY-74 and WY-75 yeast were grown to saturated overnight cultures in his-leu- defined minimal medium, and rediluted to OD₆₀₀ <0.05 for growth curves. Growth curves were performed as shown in the text, with readings taken at 90 min intervals for 12 h, and at less frequent intervals up to 48 h or longer. For colony formation assays in stationary phase, serial dilutions were made from the same cultures used for the growth curve and plated YPD plates that were grown at 30°C for 3 days, at which time colonies were counted. For tests of resistance to heat shock, cells from fresh overnights were plated onto his-leu- defined minimal plates, and grown at 42°C for periods of 0–7 h before being returned to 30°C for 3 days, after which time viable colonies were counted. For growth studies in SLAHGR medium, CGx75 yeast transformed with plasmids as indicated in RESULTS were grown to saturated overnights in trp- glucose-defined minimal medium, diluted to OD₆₀₀ <0.05 in SLAHGR, and grown as described above, with readings taken as indicated. Colony formation assays to assess viability were performed as described above. All growth curves were performed at least four times.

Interaction Analysis

EGY48 yeast was transformed by standard methods (Ito *et al.*, 1983) with plasmids expressing LexA-fusions, activation-domain fusions, or both, together with the LexA operator-LacZ reporter SH18-34 (a gift of Steve Hanes, Wadsworth Institute, Albany, NY).

For all fusion proteins, synthesis of a fusion protein of the correct length in yeast was confirmed by Western blot assays of yeast extracts (Samson *et al.*, 1989) using polyclonal antiserum specific for LexA (Brent and Ptashne, 1984) or for hemagglutinin (Babco, Richmond, CA), as appropriate. LexA-RPB7 and LexA-*hsRPB7* were expressed to comparable levels. Activation of the LacZ reporter was determined as previously described (Brent and Ptashne, 1985); β-galactosidase assays were performed on three independent colonies, on three separate occasions, and values for particular plasmid combinations varied less than 25%. Activation of the LEU2 reporter was determined by observing the colony-forming ability of yeast plated

on complete minimal medium lacking leucine. The LexA-PRD/HD expressing plasmid has been described (Golemis and Brent, 1992).

Cell Labelling and Immunoprecipitation

WY-74 and WY-75 yeast cells were labeled with [³⁵S]methionine for 1 h and immuno-precipitated as described (Kolodziej and Young, 1991), using SWG16 antibody specific to the carboxy terminal domain of the RPB1 subunit (Thompson *et al.*, 1990; Treich *et al.*, 1992). Immunoprecipitated subunits were resolved by electrophoresis on a 12% sodium dodecyl sulfate-polyacrylamide gel. Gels were fixed in 30% methanol, 10% glacial acetic acid, treated with Enlightning (Dupont, Wilmington, DE), and exposed on Kodak AR film for 1–2 days. Additional quantitation was done using a Fuji Bioanalyzer.

Northern Analysis

The synthetic oligonucleotide EG53 (AGC AAG TTG GGG CCG AAG) was used as a probe for hsRPB7 expression. The synthetic oligonucleotide EG33 (CAG TTC ATC CGG ACT CAG GAC TCC G) was used as a probe for hsRPB1 expression. Oligos were radioactively labeled using T4 polynucleotide kinase and crude [³²P]ATP (ICN Radiochemical, Cleveland, OH). The multiple tissue Northern blots, MTN1 and MTN2, were purchased from Clontech (Palo Alto, CA) and had been previously normalized to within threefold variation of mRNA levels using an actin probe. Additionally, we have stripped and rehybridized them with a probe for another gene, HEF3 (Golemis, unpublished data), and observed less than threefold variation in signal intensity between lanes. Hybridizations were carried out as described in (Ausubel *et al.*, 1987–1994). Selective temperature washes were done at 50°C; blots were exposed on Kodak AR film at –70°C for 2–10 days, and were subsequently quantitated using a Fuji Bioanalyzer.

Generation of Polyclonal Antibodies

hsRPB7 was excised from vector JG4–4 using *EcoRI* and *XhoI*, and cloned into similarly restricted plasmid pGEX (Pharmacia, Piscataway, NJ) to create a glutathione-S-transferase (GST) fusion protein. This plasmid was transformed into bacterial strain CAG456, and expression of protein was induced by standard means with IPTG (Ausubel *et al.*, 1987–1994). Induced bacteria were pelleted, resuspended in 2× Laemmli sample buffer (10% β-mercaptoethanol, 6% SDS, 20% glycerol, and 0.2 mg/ml bromophenol blue) and lysed by sonication, and the GST-hsRPB7 fused protein purified by resolution on a 10% polyacrylamide gel. The GST-hsRPB7-fused protein was visualized by aqueous Coomassie blue staining, excised from the gel, and used to inject two rabbits, following a standard schedule of boosts and bleeds (Ausubel *et al.*, 1987–1994).

Western Analysis

Yeast lysates were prepared from logarithmically growing cultures as previously described (Samson *et al.*, 1989). For mammalian cell lysates, HeLa human cervical carcinoma cells (ATCC CCL2), 293 human primary embryonal kidney cells (ATCC CRL 1573), Jurkat human acute T cell leukemia cells (ATCC TIB152), Huh6 and Huh7 human hepatoma cells (Nakabayashi *et al.*, 1982), passage 10 foreskin fibroblast cells (Repository No. GM08333A; Weiss *et al.*, 1989), and PANC-1 pancreatic epithelial carcinoma (Lieber *et al.*, 1975) were grown in recommended standard media to ~80% confluence. Adherent cells were trypsinized to remove them from plates. Cells from a single 10-cm plate were pelleted, and resuspended in 300 μl ice cold 0.25 M Tris-HCl, pH 7.4. Two hundred microliters of this suspension was immediately removed and mixed with 50 μl of 8× Laemmli sample buffer, frozen in a dry ice ethanol bath, and stored at –70°C until use. The remaining 100 μl of suspension cells were lysed by three freeze-thaw cycles, and used for quantitation of protein concentration by Lowry assay (Lowry *et al.*, 1951). Lysates frozen in 2× Laemmli buffer were thawed, sonicated briefly, and 50

μg of protein for each cell line were resolved on two SDS-polyacrylamide gels. One gel was blotted to nitrocellulose, and used for Western analysis. Visualization of RPB7 and hsRPB7 proteins was performed using 1:2000 dilutions of rabbit polyclonal antibodies to either LexA or hsRPB7 or preimmune serum from GST-hsRPB7-injected rabbits as a primary antisera (as described in RESULTS), and a 1:1000 dilution of alkaline-phosphatase-conjugated goat anti-rabbit antibodies (Sigma, St. Louis, MO) as a secondary detection system. The second gel was stained with Coomassie blue, and used to confirm that comparable quantities of proteins had been loaded, and that no protein degradation had occurred. Gels were 10% or 12% polyacrylamide, as described in RESULTS.

RESULTS

Overexpression of hsRPB7 Influences *S. cerevisiae* Cell Morphology

To identify proteins that regulate the morphology and polarity of human cells, we screened a human cDNA library for genes that enhanced formation of pseudohyphae when expressed in the yeast *S. cerevisiae*. *S. cerevisiae* undergoes a dimorphic shift in response to severe nitrogen limitation that involves changes in budding pattern, cell cycle control, cell elongation, and invasive growth into agar (Gimeno *et al.*, 1992). A galactose-inducible HeLa cell cDNA library was used to transform a yeast strain that can form pseudohyphae on nitrogen-restricted media, and a number of human genes that specifically enhanced pseudohyphal formation were identified. One of the cDNAs derived from this screen was found to cause longer filamentous projections of diploid yeast grown on low-nitrogen media, and to lead to the production of a greater number of projections per microcolony (Figure 1). In contrast, expression of this cDNA had no effect on cell morphology in haploid yeast, or in yeast grown on rich media (our unpublished results). Analysis of the sequence of this gene (Figure 2) revealed that it was a novel human gene with strong sequence similarity to the yeast RPB7 gene (McKune *et al.*, 1993). We designated this gene hsRPB7.

hsRPB7 Belongs to a Closely Related Group of RNA Polymerase Subunits

The cDNA encoding hsRPB7 consisted of 775 bp including 28 bp of 5' untranslated sequence, an ATG leading into an open reading frame of 172 amino acids, and a 25-bp polyA-tail (Figure 2). The predicted isoelectric point of the hsRPB7 protein occurs at pH 5.3, and reflects the presence of acidic residues predominantly located at the C-terminal end of the protein. Inspection of the protein sequence reveals a number of good potential phosphorylation sites for casein kinase II, as well as two potential phosphorylation sites for protein kinase C, although whether these sites are phosphorylated *in vivo* remains an open question. The yeast RPB7 subunit does not appear to be phosphorylated (Kolodziej *et al.*, 1990), and diverges from

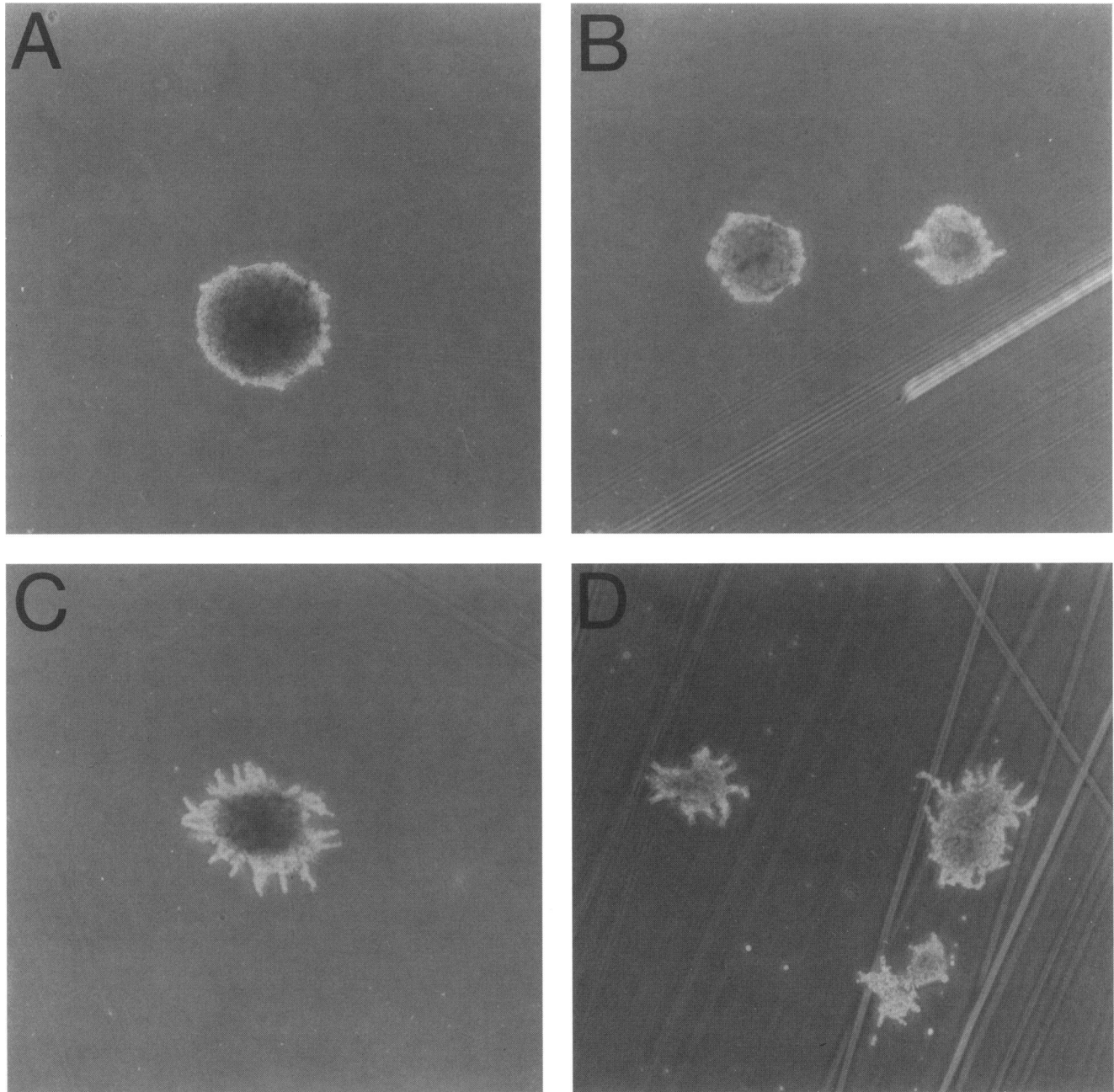


Figure 1. Colony morphology of diploid CGx74 yeast containing (A and B) JG4-4 vector or (C and D) hsRPB7 in JG4-4 streaked on SLAGHR media. Magnification 20 \times .

hsRPB7 at most of the possible casein kinase II and protein kinase C consensus sites. hsRPB7 sequence information has been filed with GenBank (accession number U20659).

Comparison of the predicted hsRPB7 coding sequence to the GenBank database using the BLAST algorithm (Altschul *et al.*, 1990) revealed significant homology to a family of RNA polymerase subunits of

which the most well characterized member is the yeast RPB7 subunit (McKune *et al.*, 1993) (Figure 3, A and B). hsRPB7 possesses 43% identity (63% similarity) to RPB7, and 53% identity (76% similarity) to the fifth largest subunits of the RNA polymerases of *Glycine max* (soybean) and *Arabidopsis thaliana* (Ulmasov and Guilfoyle, 1992). Comparison of the yeast, plant, and human sequences also indicates that the human se-

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ACTCTGCCTGCCTACCTGGTCTGGGAAGATGTTCTACCATATCTCCCTAGAGCACGAAAT
1  M F Y H I S L E H E I
CCTGCTGCACCGCGCTACTTGGGCCCACTTGCTCAACACGGTGAAGCAGAAGTCTT
12 L L H P R Y F G P N L L N T V K Q K L F
CACCGAGGTGGAGGGACCTGCACAGGGAAGTATGGCTTTGTAATTGCTGTCAACCACAT
32 T E V E G T C T G K Y G F V I A V T T I
TGACAATATTGGTGTGTGATCCAGCCAGGCGAGGCTTTGTCCTTTATCCAGTTAA
52 D N I G A G V I Q P G R G F V L Y P V K
GTACAAGGCCATTGTTTTTCGGCCATTAAAGGGGAGTTCGTGGATGCTGTGCTACTCA
72 Y K A I V F R P F K G E V V D A V V T Q
GGTCAACAAGGTGGACTCTTACAGAAATGGGCCCATGTCTTGCTTCATCTCTCGACA
92 V N K V G L F T E I G P M S C F I S R H
TTCCATCCCTTCAGAGATGGAGTTTGATCCTAACTCCAACCCACCATGTTACAAGACAAT
112 S I P S E M E F D P N S N P P C Y K T M
GGATGAGGATATTGTGATTGATCAGCAGGACGATGAGATCCGCTTAAAGATTGTGGGGACCG
132 D E D I V I Q Q D D E I R L K I V G T R
TGTGGACAAGAATGACATTTTGTCTATTGGCTCCCTGATGGACGATTACTTGGGGCTTGT
152 V D K N D I F A I G S L M D D Y L G L V
AAGCTGAGCCTGGTGGCCCTCTACCTTGGTCTACTCTAGGAAGTGTGATTGTCACACT
172 S *
TATCATGTTGTCCAGAGGTCCAGTCTGGCTGCTGTTGTGGAGGCAAGGAAGCAATCAT
CCCAGAAGGCATCTGGTGCTCTTGTAGCTTAAGTCTGCTCCTCATTTCAGTATGT
GTTCTAAGTATAAAAAGTCCTTTGGTTCTCAAAAAAAAAAAAAAAAAAAAAA

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Figure 2. Sequence of the *hsRBP7* cDNA and predicted amino acid sequence of the *hsRBP7* subunit. cDNA excluding polyA tail is 750 bp, and predicted ORF is 172 amino acids. The protein sequence contains six potential casein kinase II sites at amino acid positions 32, 49, 115, 130, 150, and 162, as well as two potential protein kinase C sites at positions 25 and 39.

quence is closer to yeast and plant than they are to each other. Although more distantly related, *hsRBP7* also possessed 21% identity (48% similarity) to the yeast *YKL1* gene (Abraham *et al.*, 1992), which has recently been shown to encode the C25 subunit of yeast RNA polymerase III (Pati and Weissman, 1990; Sadhale and Woychik, 1994), and 21% identity (44% similarity) to the *rpoE* subunit of the archaeobacterium *Sulfolobus acidocaldarius* (GenBank accession number X75411). The conservation of sequence through such an evolutionarily divergent group of organisms suggests an important function for the *RBP7* family of proteins. Finally, although the sequence similarity between this group of proteins extends through the entire length of sequence, it is most pronounced in the region extending from amino acid residues ~70–90 of the *hsRBP7* sequence. This conserved domain is essential for *RBP7* and C25 function in *S. cerevisiae* because deletion of this region resulted in lethality (Sadhale and Woychik, 1994), and likely serves an important role in human cells as well.

Overexpression of *RBP7* also Alters *S. cerevisiae* Cell Morphology, but Overexpression of *RBP4* Does Not

Because we were surprised to isolate a pol II subunit in a screen expected to yield regulators of cell morphology, we wanted to verify the physiological signif-

icance of this result by testing whether *RBP7* could similarly enhance pseudohyphae in yeast. We overexpressed the yeast *RBP7* gene from the same galactose-inducible expression vector used to clone *hsRBP7*, and compared pseudohyphal formation by *hsRBP7*, *RBP7*, or vector-containing CGx74 (Figure 4). Cell elongation and adaptation of a unipolar budding pattern was notably more pronounced with the yeast *RBP7* than with the human *hsRBP7*, suggesting that the phenotype was not the result of *hsRBP7* interfering with *RBP7* function.

RBP7 has been shown to form a highly stable subcomplex with the yeast *RBP4* subunit (Edwards *et al.*, 1991; Young, 1991). This subcomplex appears to play a key role in enabling yeast survival during periods of stress and during stationary phase (Choder, 1993; Choder and Young, 1993). As *RBP4* function might potentially be relevant for growth on the severely nitrogen-limited medium used to establish pseudohyphal growth, we tested whether overexpression of *RBP4* by itself was sufficient to induce pseudohyphal growth, or whether co-expression of *RBP4* with *hsRBP7* or *RBP7* would affect pseudohyphal growth induced by these genes (Figure 5 and our unpublished results). We found that *RBP4* overexpression did not by itself induce expression of pseudohyphae (Figure 5, A and D). Further, co-expression of *RBP4* with *RBP7* (Figure 5, E and H) or *hsRBP7* (our unpublished results) neither enhanced nor reduced pseudohyphal induction by *RBP7*.

***hsRBP7* Can Complement *RBP7* Deletion Strains at Moderate Temperatures**

Because the amino acid sequence of the *hsRBP7* protein is well conserved from humans to yeast, we wanted to determine if *hsRBP7* can functionally substitute for *S. cerevisiae* *RBP7*. Using the plasmid shuffle method (Boeke *et al.*, 1987), we tested whether expression of *hsRBP7* was able to rescue the lethality associated with an *RBP7* null mutation (McKune *et al.*, 1993). Yeast cells that have the chromosomal copy of *RBP7* disrupted by insertion of the *LEU2* gene plus a wild-type complementing copy of the *RBP7* gene on a *URA3* plasmid were transformed with an additional *HIS3* plasmid, containing either *hsRBP7* under the control of the *RBP7* promoter or the *RBP7* gene and its promoter. *Ura*⁺*His*⁺*Leu*⁺ transformants were transferred to media containing 5-fluoroorotic acid to select for loss of the yeast *RBP7/URA3* plasmid. Both the yeast *RBP7* control plasmid and the *hsRBP7* plasmid supported wild-type levels of yeast cell growth at moderate temperatures (Figure 6A), indicating that the human *RBP7* homologue can functionally replace its yeast counterpart under normal growth conditions.

hsRPB7-dependent Yeast Cells Are Temperature Sensitive, Cold Sensitive, and Have Stationary Phase Defects

Induction of pseudohyphal growth occurs in response to nutritional stress (i.e., nitrogen restriction). We tested the growth of yeast containing only hsRPB7 in response to other stressors, including high temperature, low temperature, heat shock, and maintenance at stationary phase. In contrast to their growth at moderate temperatures, yeast cells expressing only hsRPB7 possessed phenotypes strikingly different from wild-type yeast when cultured at high and low temperatures, or when continuously cultured after reaching stationary phase. After shifting cells from 30° to 37°C, yeast expressing hsRPB7 grow through early and mid-

logarithmic phase at essentially the same rate as wild-type yeast. As they enter late log phase, the growth rate of hsRPB7 cultures is somewhat reduced relative to that of wild-type yeast, and cultures saturate at lower levels (Figure 6A). Observation of the cells in the hsRPB7 and RPB7 cultures at 37°C indicated that although cells in both cultures rapidly became morphologically abnormal, with many cells developing vacuoles, "bumpy" cell walls, and misformed buds, the abnormalities were more severe in yeast containing only hsRPB7. Removal and replating of equivalent numbers of cells from stationary phase cultures of hsRPB7 and RPB7 yeast grown at 37° and 30°C indicated that hsRPB7 yeast lost viability significantly more rapidly than RPB7 yeast at both temperatures,

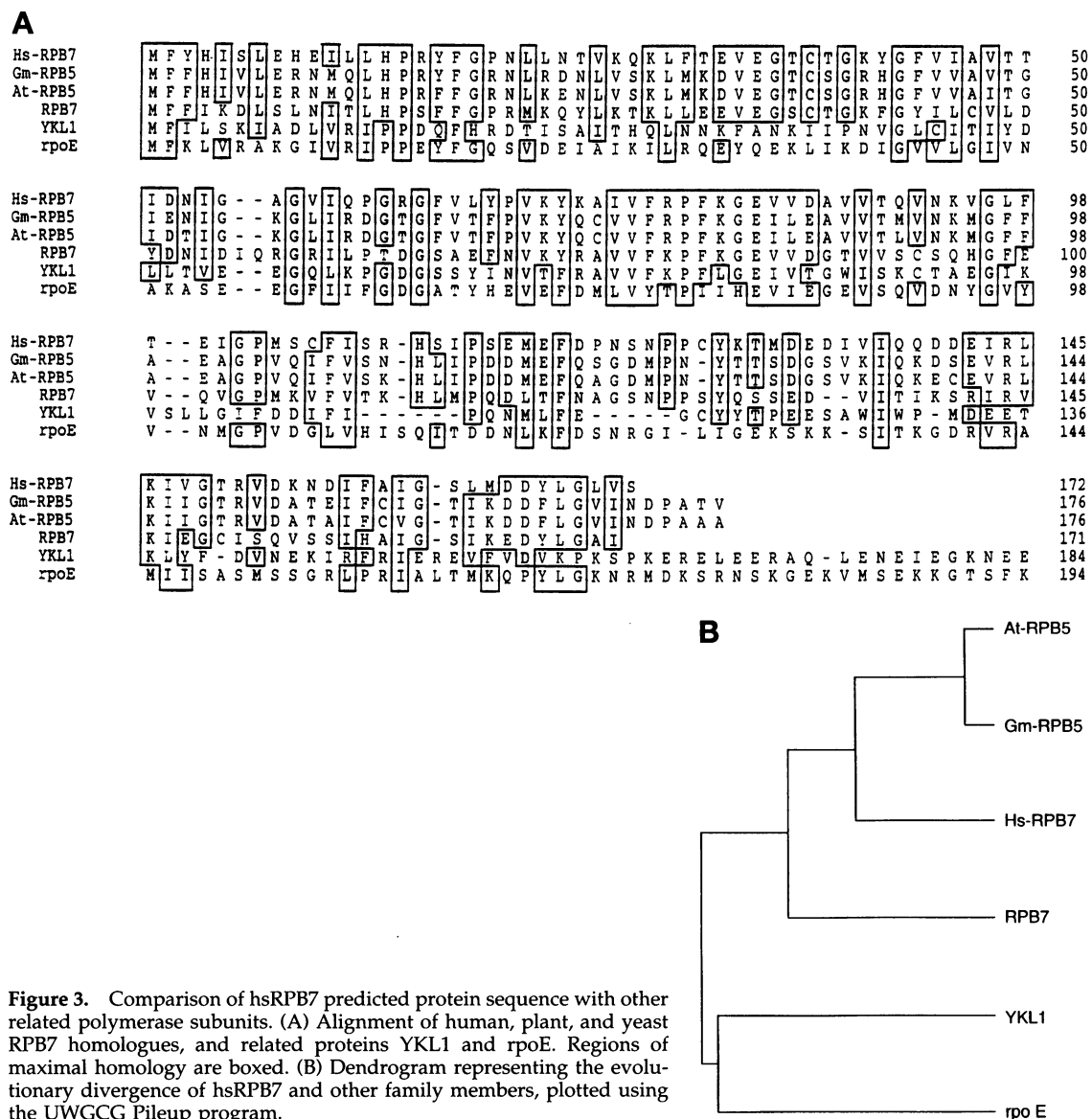


Figure 3. Comparison of hsRPB7 predicted protein sequence with other related polymerase subunits. (A) Alignment of human, plant, and yeast RPB7 homologues, and related proteins YKL1 and rpoE. Regions of maximal homology are boxed. (B) Dendrogram representing the evolutionary divergence of hsRPB7 and other family members, plotted using the UWGCG Pileup program.

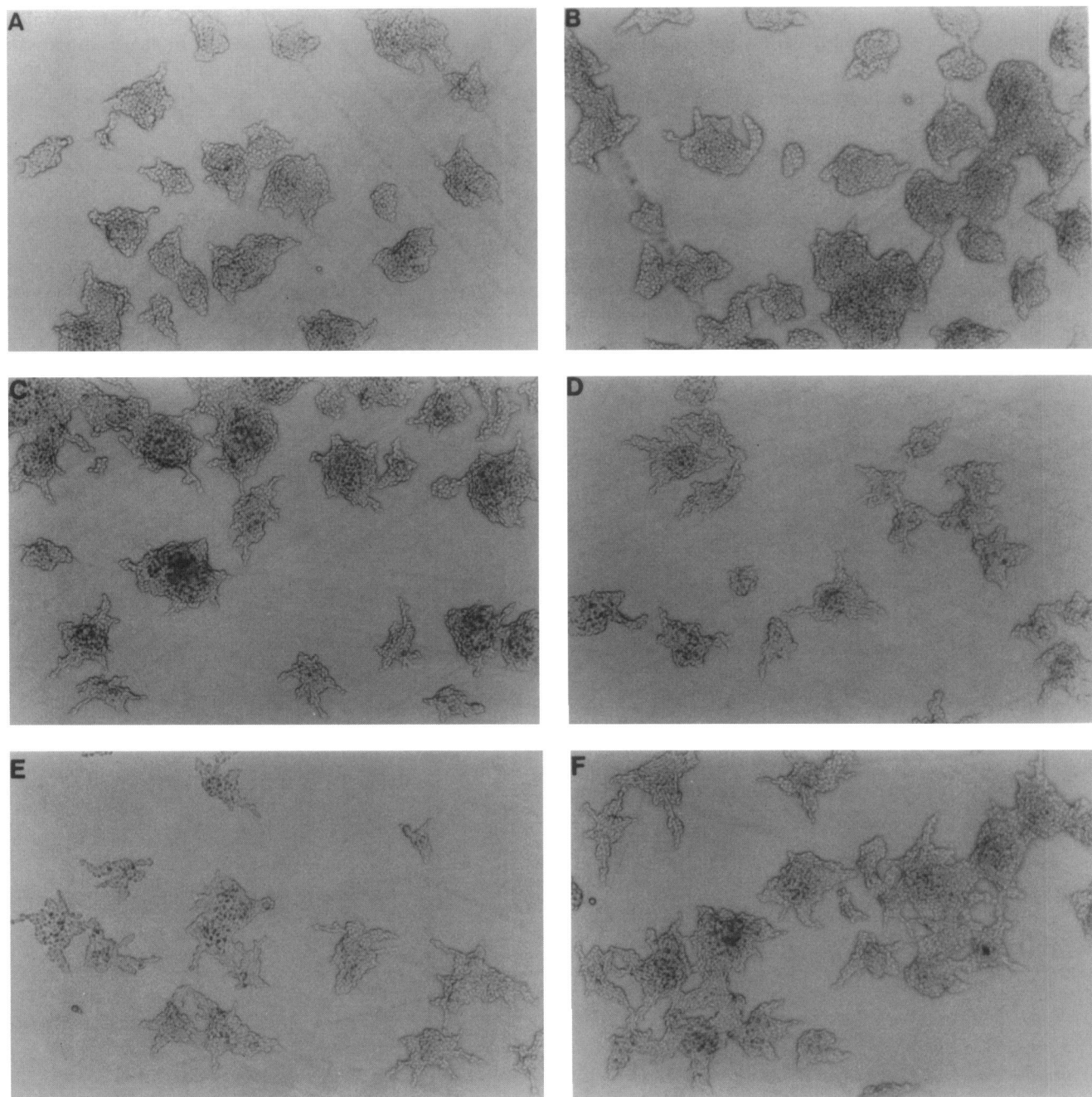


Figure 4. Colony morphology of diploid CGx74 yeast containing pJG4-4 vector (A and B), pJG4-4/RPB7 (C and D), or pJG4-4/RPB7 (E and F) streaked on SLAGHR medium. Colonies were photographed after 36 h. Magnification 200 \times .

with the difference most pronounced at 37°C (Figure 6B). hsRPB7 yeast also grew more poorly than RPB7 yeast at 12°C, although the difference was less pronounced.

Although overexpression of RPB4 did not affect enhancement of pseudohyphal growth by RPB7, the defects seen in hsRPB7 yeast cells, including conditional

Figure 5 (facing page). Colony morphology of diploid CGx75 yeast containing pJG4-4 vector and pYES2 vector (A and B), pJG4-4 and pYES/RPB4 (C and D), pJG4-4/RPB7 and pYES (E and F), and pJG4-4/RPB7 and pYES/RPB4 (G and H), streaked on SLAGHR medium. Colonies were photographed after 72 h (A, C, E, and G) or 24 h (B, D, F, and H) growth at 30°C. Magnification 200 \times .

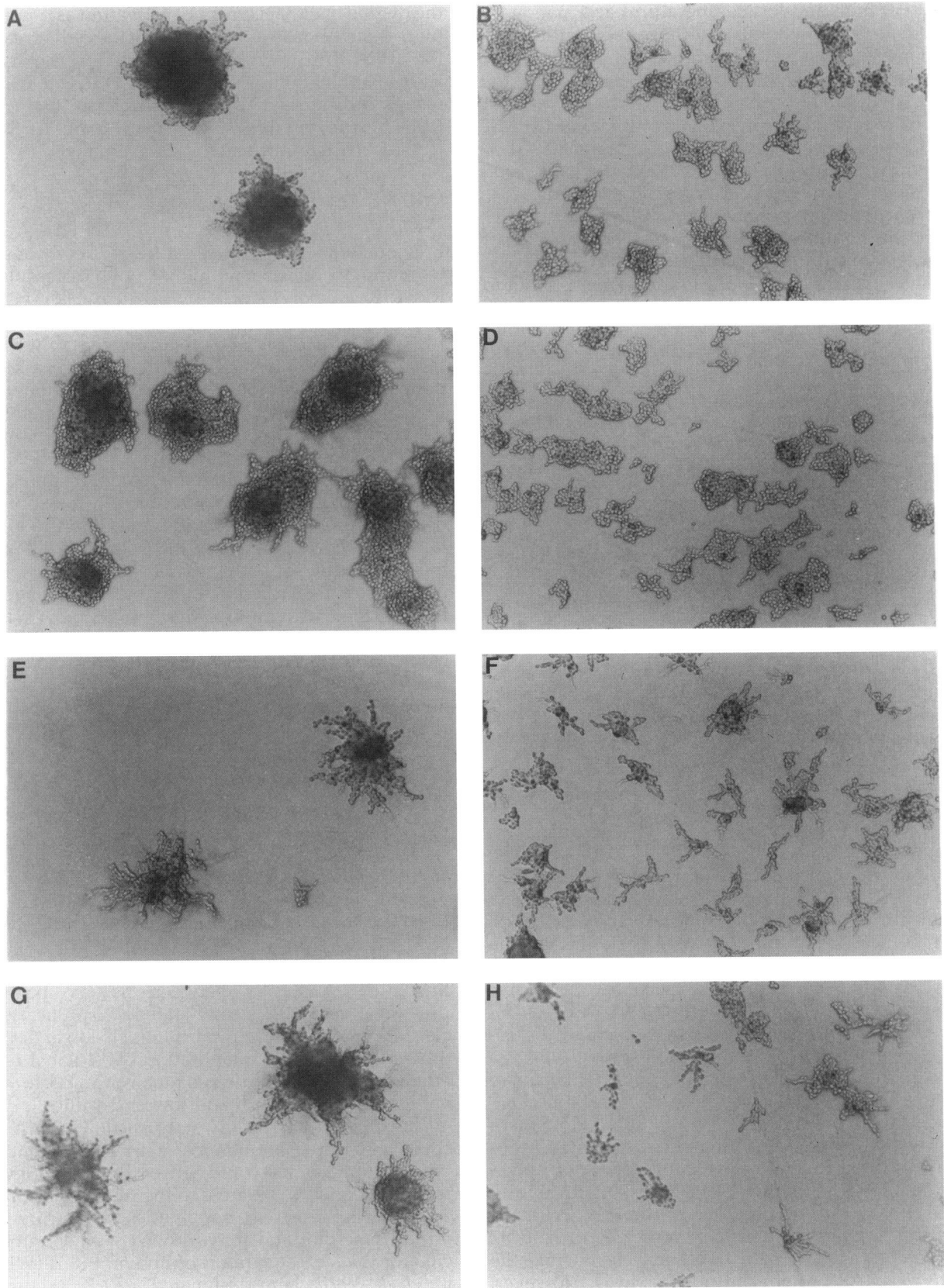


Figure 5.

lethality at high and low temperatures and stationary phase defects, were similar to those seen with *rpb4*⁻ mutations (Choder and Young, 1993). We therefore tested an additional parameter associated with the lack of the *RPB4* gene, reduced resistance to heat shock (Choder and Young, 1993). Cultures of hsRPB7 and RPB7 expressing yeast were grown at 30°C, then exposed to timed heat shock at 42°C. Yeast expressing RPB7 remained fully viable for at least 7 h at 42°C, whereas yeast expressing hsRPB7 rapidly lost viability and became completely inviable between 3 and 5 h of heat shock (our unpublished results).

Because overexpression of RPB4 has been shown to increase growth rate of post-logarithmic growth phase cultures (Choder, 1993), we wanted to test whether overexpression of hsRPB7 or RPB7 affected growth rates as well. If so, these genes might be contributing to pseudohyphal development by stimulating cell growth. We compared growth rate at 30°C in SLAHGR (low nitrogen) medium of CGx74 yeast overexpressing hsRPB7 or RPB7 to that of control CGx74 yeast expressing JG4-4 vector (Figure 7). Lag time before commencement of cell division, doubling time, and saturation density were similar for all three cultures: overexpression of RPB7 may have marginally decreased growth rate, although the decrease was within the limit of experimental variation. In contrast to the loss of viability observed after long-term culture in yeast growing under control of hsRPB7 (Figure 6B), yeast overexpressing hsRPB7 and RPB7 maintained viability similar to control yeast after incubation for >96 h in SLAGHR medium. These data exclude a role for overexpression of RPB7 in increasing growth rate or inducing severe toxicity on low nitrogen medium.

hsRPB7 Associates with Yeast RPB4

The above data indicated that hsRPB7 could complement the essential function of RPB7, but might be deficient in its ability to interact with RPB4 or another protein in the transcription complex. To directly test this hypothesis, we utilized a two-hybrid/interaction trap approach (Fields and Song, 1989; Gyuris *et al.*, 1993; Golemis *et al.*, 1994). We used the constitutive ADH promoter to express hsRPB7 and RPB7 as fusions to the DNA binding protein LexA, and the galactose-inducible GAL1 promoter to express RPB4 as a fusion to a transcriptional activation domain. We transformed these constructions and a negative control (LexA fused to the PRD box and homeodomain of bicoid (Gyuris *et al.*, 1993)) together with a LexA operator-LacZ reporter into yeast, and compared activation of the reporter by LexA-RPB7, LexA-hsRPB7, and LexA-PRD/HD in the presence or the absence of the activation domain-fused RPB4 protein. Both hsRPB7 and RPB7 interacted with RPB4 in yeast, while the negative control protein did not (Table 1). However,

the degree of enhancement of activation as quantitated by β -galactosidase assay was considerably greater for the RPB4-RPB7 combination than for the hsRPB7-RPB4 combination (60-fold versus 2- to 3-fold). These results indicate that hsRPB7 is able to interact with RPB4 *in vivo*, but does so with a lower affinity than the RPB4-RPB7 subunit pair.

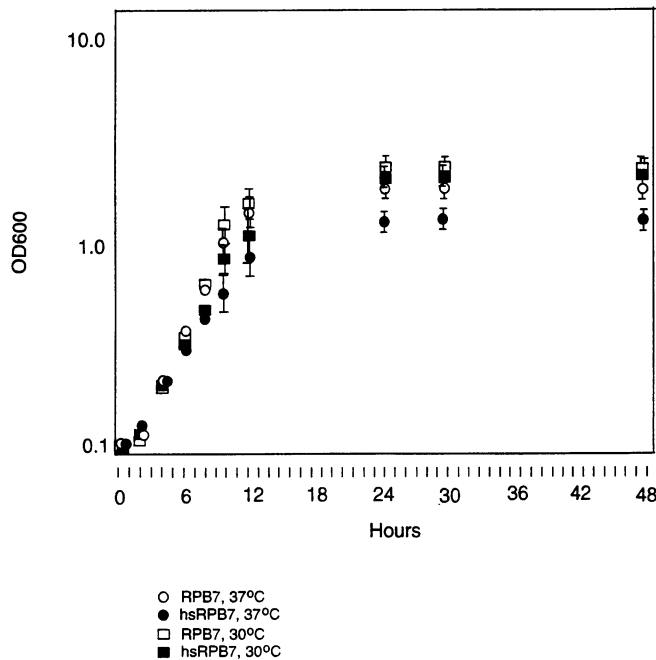
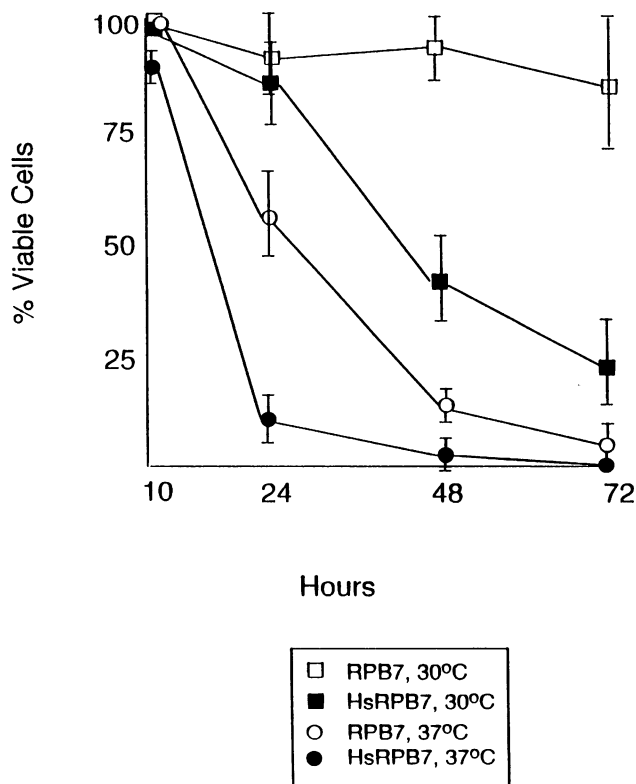
hsRPB7 Assembles with Yeast RNA Polymerase II Subunits

Previous work has shown that RPB7 does not appear to immunoprecipitate with RNA pol II in yeast lacking RPB4 (Kolodziej *et al.*, 1990). Because *rpb7*⁻ yeast are inviable, it has been impossible to determine whether RPB7 is required for RPB4 to associate stably with pol II. Because our data suggested that hsRPB7 associated poorly with RPB4, one possible explanation for the *rpb4*⁻-like defects of hsRPB7 yeast was that RPB4/hRPB7 was unable to assemble with the remainder of the pol II subunits. Alternatively, hsRPB7/RPB4 could associate with the remainder of the pol II subunits, but result in a complex that was not fully functional. To discriminate these possibilities, we immunoprecipitated ³⁵S-labeled pol II from RPB7- or hsRPB7-expressing yeast using an antibody to the carboxy terminal domain of the largest subunit of pol II (Figure 8), resolving individual subunits by SDS-polyarylamide gel electrophoresis (PAGE). Quantitation of the stoichiometry of precipitated subunits from the two species was identical, indicating that hsRPB7 is able to assemble as efficiently as its yeast counterpart with the full set of yeast pol II subunits, including RPB4.

Tissue-specific Expression of hsRPB7 RNA

We examined the expression levels of hsRPB7 mRNA in different human tissues using Northern blot analysis (Figure 9A). The *hsRPB7* probe hybridized to a single band of approximately 800 bp, which together with the presence of homology to the full length of RPB7 coding sequence indicates that the hsRPB7 cDNA isolated in the screen is full length or close to full length. Surprisingly, however, the *hsRPB7* mRNA fluctuated drastically between tissues, being expressed at very high steady-state levels in heart, muscle, kidney, and liver, and at much lower levels in other tissues. Direct quantitation of ³²P label using a Fuji Bioanalyzer indicated as much as an 80- to 90-fold variation between high and low end values.

This difference in steady-state *hsRPB7* mRNA levels could reflect the abundance of transcripts encoding pol II subunits, or it could reflect differential levels of the *hsRPB7* mRNA relative to the rest of the complex. To resolve these two possibilities, we stripped the multi-tissue blot and rehybridized with a probe specific for the largest subunit of human pol II (Wintzerith *et al.*, 1992), here designated *hsRPB1* (Figure 9B).

A Growth curve at 30°C and 37°C, hsRPB7 and RPB7**B** Viability at 30°C and 37°C, hsRPB7 and RPB7

This probe hybridized specifically to a single transcript of ~6.7 kb, corresponding to the reported size of the transcript encoding this subunit (Wintzerith *et al.*, 1992), that was present at similar levels in all tissues examined. Interestingly, placental tissue contained a single transcript of ~7.5–8 kb, which might represent an alternately spliced form of the gene encoding the largest subunit. Strikingly, the steady-state levels of the *hsRPB1* transcript differ substantially in tissue distribution relative to the *hsRPB7* transcript. Although levels of *hsRPB1* were somewhat elevated in kidney and muscle (although much less than the *hsRPB7* transcript), they were extremely low in liver and heart. These data suggest that transcription or stability of the *hsRPB7* mRNA is regulated differently than that of the largest pol II subunit, and imply that *hsRPB7* might be differentially available to RNA polymerase II in different tissues.

Expression and Modification of hsRPB7 Protein

The yeast RPB7 protein migrates as a 17–18 kDa species on SDS-PAGE gels (Sentenac, 1985), and is not post-translationally modified (Kolodziej *et al.*, 1990). We examined expression of the *hsRPB7* protein in yeast and human cells using polyclonal antiserum raised against a GST-*hsRPB7* protein fusion (Figure 10). To confirm that this antiserum reacted specifically with *hsRPB7* but not with RPB7, we used 10% polyacrylamide gels to resolve whole cell lysates of cells expressing either native RPB7 or *hsRPB7* proteins, or LexA-fused RPB7 or *hsRPB7*. We probed triplicate blots of these resolved lysates with either antibody to LexA, antibody to GST-*hsRPB7*, or pre-immune serum from GST-*hsRPB7* rabbits (Figure 10A).

Antibody to LexA revealed similar expression levels of LexA-*hsRPB7* and LexA-RPB7 fusion proteins (Figure 10A, left two lanes in each panel), with LexA-*hsRPB7* migrating with a slightly higher molecular weight than LexA-RPB7. In contrast, antibody to *hsRPB7* specifically detected LexA-*hsRPB7*. Using the same panel of antisera on WY74 (RPB7 only) and WY75 (*hsRPB7* only) yeast extracts (Figure 10A, right two lanes of each panel), antibody to *hsRPB7* detected a single protein migrating at approximately 24 kDa in lanes containing WY75 extracts, whereas neither of the other antisera recognized any specific proteins. In con-

Figure 6. Growth properties of yeast with RPB7 replaced by *hsRPB7*. (A) Growth rate at 30°C and 37°C. WY-74 yeast containing RPB7 (open squares) or WY-75 yeast containing *hsRPB7* (closed squares) growing at 30°C and WY-74 yeast expressing RPB7 (open circles) or WY-75 yeast expressing *hsRPB7* (closed circles) growing at 37°C. (B) Viability at stationary phase. WY-74 (RPB7) and WY-75 (*hsRPB7*) yeast were maintained in his-leu- defined minimal media at 30°C and 37°C for 3 days; key as in Figure 4. Equivalent numbers of cells were plated to YPD media, and grown for 3 days at 30°C to determine numbers of viable cells remaining in the culture.

Growth curve in SLAHGR with overexpressed RPB7 or hsRPB7

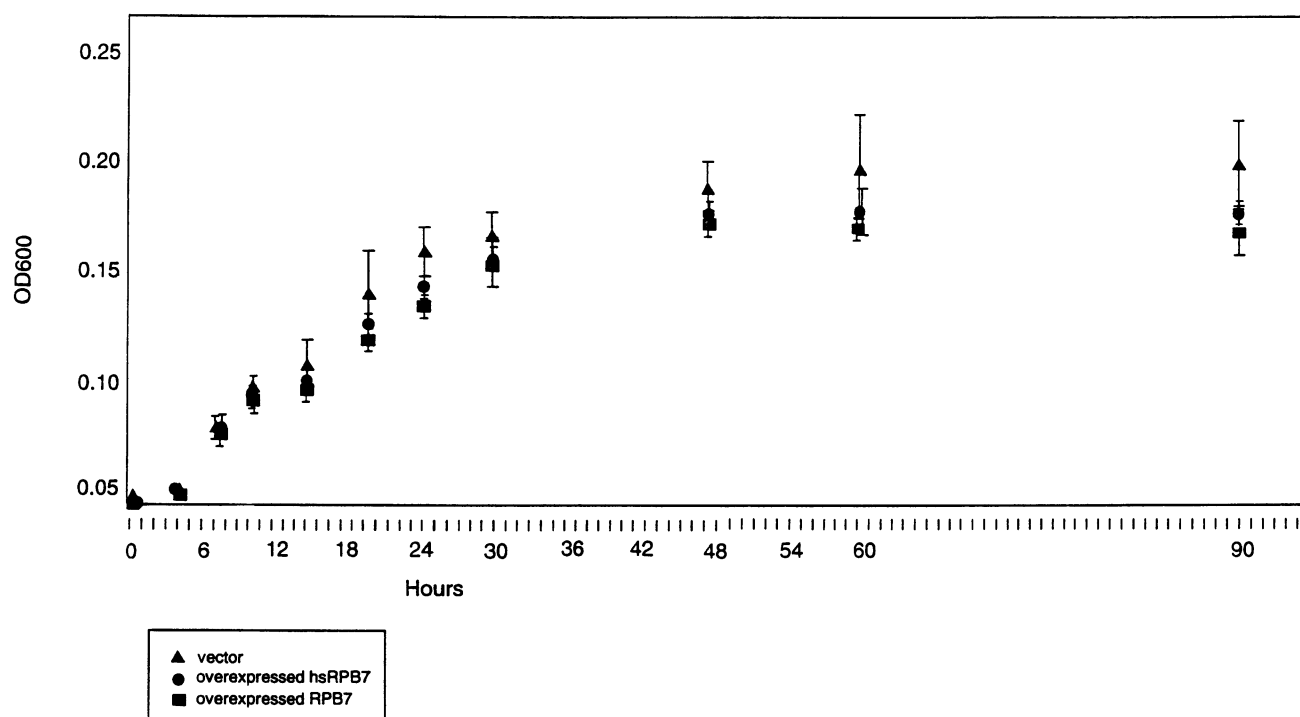


Figure 7. Growth rate of yeast overexpressing RPB7 or hsRPB7 in SLAHGR medium. CGx75 yeast containing pYES2 and pJG4-4 (closed triangles), pJG4-4/hsRPB7 (closed circles), or pJG4-4/RPB7 (closed squares) were grown at 30°C.

trast, antibody to hsRPB7 detected two specific bands, one of 24 kDa and a second of 18 kDa, in a whole cell extract of 293 human embryonal kidney cells (Figure 10A, central lane in first panel).

We then used the antiserum to hsRPB7 to compare protein expression in seven human cell lines, this time using a 12% polyacrylamide gel to increase resolution of lower molecular weight protein species (Figure 10B). In each case the antibody detected a species at 24 kDa and a species at 18 kDa. Abundance of both the 24

kDa and 18 kDa species varied significantly between different cell lines tested, with Jurkat and 293 kidney cells highest, and human foreskin fibroblasts lowest. A number of the cell lines (293, Huh6, and Huh7) possessed additional specific cross-reacting species migrating at ~17 kDa and ~14 kDa, and one cell line, Jurkat, had a broad band at ~17–18 kDa that might contain multiple species. These bands may correspond to differentially modified forms of the hsRPB7 protein, or to other related proteins.

Table 1. Interaction of RPB7 and hsRPB7 with RPB4

	+JG4-5	+JG4-5/RPB4	Induction
LexA-hsRPB7	6.9	19.6	2.8
LexA-RPB7	1.6	87.6	59.4
LexA-bic ₂₋₁₆₀	1.2	2.0	1.3

EGY191 yeast were transformed with the SH18-34 LexA-operator-LacZ reporter and the indicated combinations of LexA-fused and activation-domain-fused proteins. β -galactosidase values shown reflect the average values determined for three individual isolates for each data point; variation between isolates was less than 20%.

DISCUSSION

After identifying the *hsRPB7* gene using a screen for proteins that influence yeast cell morphology, we established that at least part of the RPB7 function has been conserved through humans, to the extent that hsRPB7 expression can rescue the lethality of an *rpb7* deletion. However, yeast expressing the hsRPB7 subunit have significantly altered growth properties relative to yeast expressing RPB7. Growth differences are particularly evident under extreme conditions, which is likely due to an altered association of hsRPB7 with the yeast RPB4 subunit. We have found that both

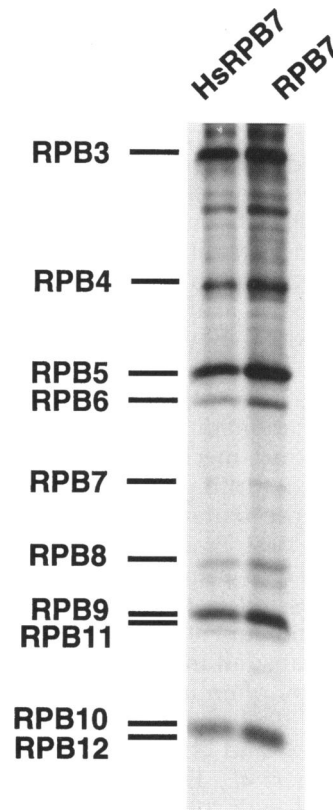


Figure 8. Immunoprecipitation of RNA polymerase II from yeast containing only RPB7 (WY-74) or only hsRPB7 (WY-75), using antibodies specific for the C-terminal domain of the largest subunit of pol II.

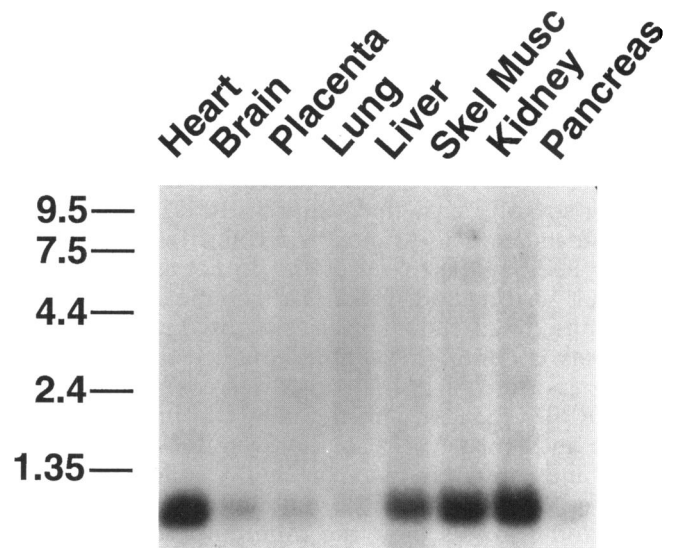
hsRPB7 and RPB7 are able to enhance formation of pseudohyphae in yeast, implying an unexpected connection between a core element of the yeast transcription apparatus and a change in cell growth control. The fact that the expression pattern of *hsRPB7* in humans is variable between different tissues suggests this subunit may play a regulatory role, but this remains to be established.

Human homologues have been reported previously for a number of the twelve subunits of the *S. cerevisiae* RNA polymerase II (Cho *et al.*, 1985; Pati and Weissman, 1989; Pati and Weissman, 1990; Acker *et al.*, 1992; Wintzerith *et al.*, 1992; Acker *et al.*, 1993; McKune and Woychik, 1994; Pati, 1994). In general, the degree of evolutionary sequence conservation is quite high for all the subunits so far cloned. Of subunits tested to date, the human homologue has been able to at least partially complement the yeast equivalent in six cases (including hsRPB7) and not been able to in one other (McKune *et al.*, 1993; Woychik, unpublished data).

Results from the two-hybrid, co-precipitation, and Western blot experiments described here suggest that a mechanism for the conditional complementation phenotype of hsRPB7 is the inability of RPB4 to complex appropriately with the 24-kDa form of hsRPB7. We derive this conclusion from the following data: 1) two-hybrid data indicate hsRPB7 and RPB4 appear to

interact less stably than RPB7 and RPB4. 2) pol II immunoprecipitated from yeast growing under the control of hsRPB7 contains levels of RPB4 and the 18-kDa form of hsRPB7 similar to the levels of RPB4

A hsRPB7



B hsRPB1

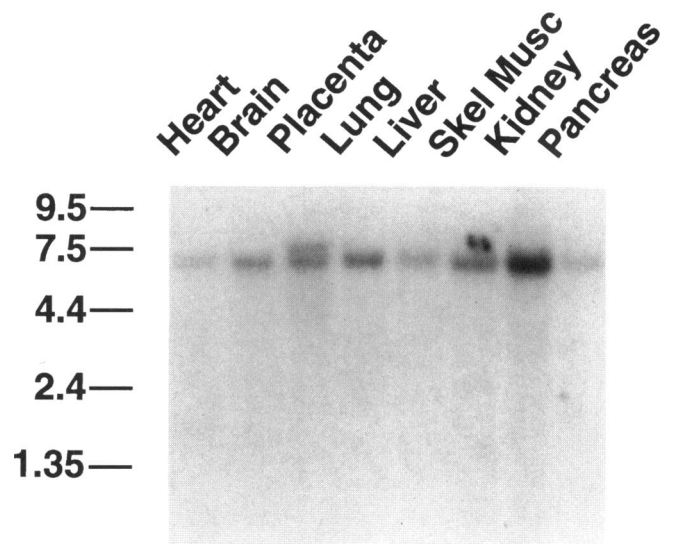


Figure 9. Northern blot analysis of hsRPB7 and hsRPB1 RNAs. The multi-tissue RNA blot was probed with labeled oligonucleotide probes specific to (A) hsRPB7 and (B) hsRPB1 as described in MATERIALS AND METHODS.

and RPB7 found in wild-type yeast, with hsRPB7 and RPB7 showing equivalent mobilities on resolving gels. In contrast, when hsRPB7 is synthesized in yeast, only a higher migrating (24 kDa) form is recognized by the hsRPB7. This 24-kDa form is probably the result of post-translational modification and appears to be the predominant form of hsRPB7 in yeast. We have excluded the possibility that our antibody preferentially recognizes this higher migrating form by control experiments in which antibody to LexA indicates LexA-hsRPB7 predominantly migrates at a higher molecular mass than LexA-RPB7. Thus, under normal growth conditions in which RPB4 function is not necessary, hsRPB7 can fulfill the essential function of RPB7, and the small quantity of unmodified hsRPB7 present (which is apparently not abundant enough to be detectable by Western analysis) can assemble into pol II. Under stressful growth conditions, RPB4 is unable to execute its protective function in concert with hsRPB7, either because the two proteins do not associate in a manner leading to a functional complex, or because the unmodified 18-kDa species with which RPB4 can complex is limiting. It should be noted that although the proportion of pol II complexes containing RPB4 and RPB7 changes substantially during stationary phase and stress response, the overall levels of RPB4 RNA and protein remain constant (Choder, 1993), indicating the RPB4 and RPB7 subunits are normally in substantial excess over the minimum concentration

required to associate with pol II. Thus, although unmodified 18-kDa hsRPB7 may represent a small proportion of the total hsRPB7 expressed in yeast, it is sufficient to bind to pol II during growth under normal conditions. An additional implication of this model would be that changes in the expression of other transcription complex-associated factors might control the enhanced recruitment of RPB4/RPB7 to pol II under conditions of stress. Whatever the nature of the modification leading to the higher form, it appears not to be an artifact specific to heterologous expression of hsRPB7 in yeast, as human cells express abundant quantities of an equivalently migrating species.

Is RPB7 actually a mediator of pseudohyphal differentiation, or does overexpression of this gene affect a distinct mechanism controlling cell morphology? To date, a number of groups have characterized genes whose mutation leads to enhancement (Ljungdahl *et al.*, 1992; Blacketer *et al.*, 1993, 1994) or loss (Liu *et al.*, 1993) of pseudohyphal expression, or whose overexpression leads to constitutive pseudohyphal expression (Gimeno and Fink, 1994). Genes isolated by these means fall into a number of classes, including a facilitator of amino acid permease transport (Ljungdahl *et al.*, 1992), kinases from the mating factor (Liu *et al.*, 1993) and other signal transduction pathways (Blacketer *et al.*, 1993), and a transcription factor (Gimeno and Fink, 1994). This diversity indicates that the process of pseudohyphal conversion is complex, and reg-

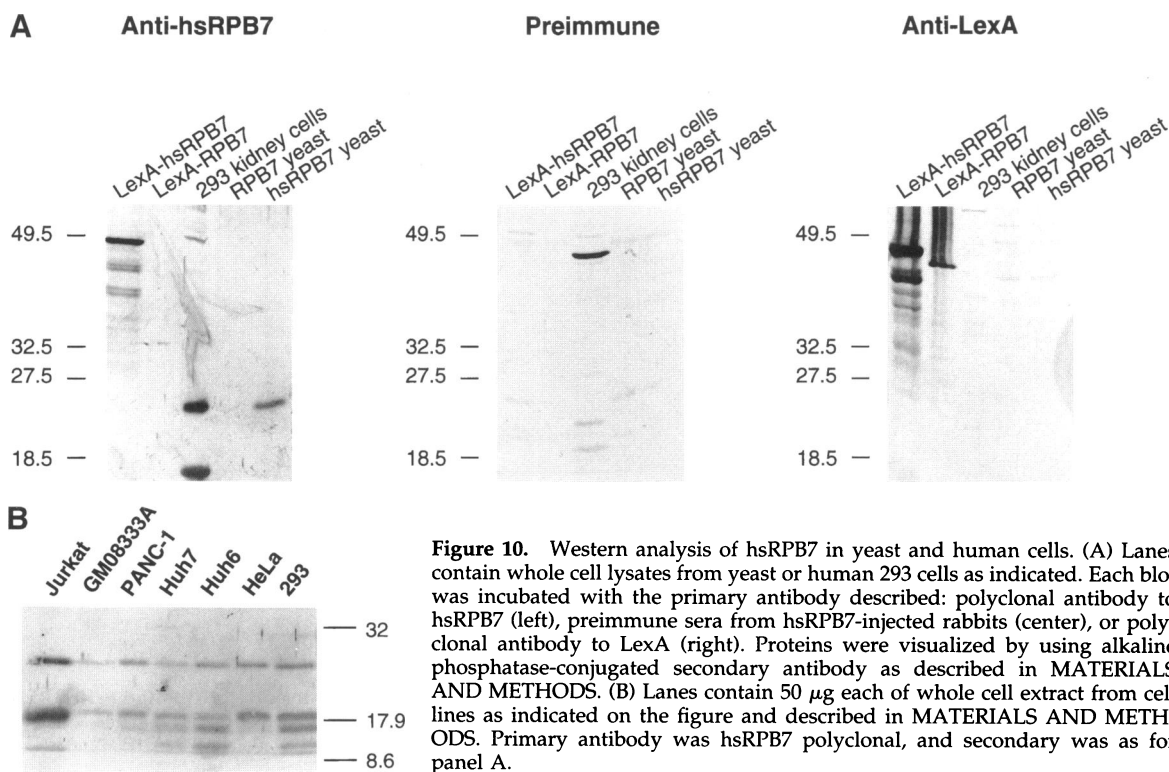


Figure 10. Western analysis of hsRPB7 in yeast and human cells. (A) Lanes contain whole cell lysates from yeast or human 293 cells as indicated. Each blot was incubated with the primary antibody described: polyclonal antibody to hsRPB7 (left), preimmune sera from hsRPB7-injected rabbits (center), or polyclonal antibody to LexA (right). Proteins were visualized by using alkaline phosphatase-conjugated secondary antibody as described in MATERIALS AND METHODS. (B) Lanes contain 50 μ g each of whole cell extract from cell lines as indicated on the figure and described in MATERIALS AND METHODS. Primary antibody was hsRPB7 polyclonal, and secondary was as for panel A.

ulated at numerous levels. While misexpression of other genes not known to be involved in regulation of pseudohyphal transition can result in cell elongation (Haarer and Pringle, 1987; Richardson *et al.*, 1990), several points seem to support a role for RPB7 as a contributory factor to pseudohyphal growth. First, the phenotype is specific to diploid yeast. Second, it is specific to growth on low-nitrogen plates. Third, the fact that the yeast RPB7 pseudohyphal effect is more pronounced than the human eliminates the possibility that cell elongation is due to a nonspecific derangement of normal growth controls because of aberrant transcription induced by pol II containing hsRPB7. Overexpression of neither hsRPB7 nor RPB7 appears to be toxic in yeast, in that yeast expressing high levels of RPB7 or hsRPB7 in a wild-type background grow as rapidly as control yeast under a variety of growth conditions. At this time, the simplest interpretation of these data is that the RPB4/RPB7 system, previously only shown to be required for stress survival, also contributes to cell elongation during pseudohyphal growth, a morphological change induced by the severe limitation of nitrogen in the growth medium (Gimeno *et al.*, 1992), and enhanced by the presence of yeast mutations that intensify this nutritional stress (Ljungdahl *et al.*, 1992).

Pseudohyphal cells have a greater cell size than cells growing normally, and are considerably elongated (Gimeno *et al.*, 1992); the combination of these changes with a conversion of budding pattern to unipolar allows colonies to spread over greater distance than normally dividing cells, a protective change that increases the likelihood of reaching a food source (Gimeno *et al.*, 1992). A potentially interesting analogy can be derived from the prokaryotic system of selectively utilizing different σ factors in developmental programs (reviewed in Shapiro, 1993) or in stress response pathways (reviewed in Hengge-Aronis, 1993). In particular, the *rpoS* gene of *E. coli* encodes σ^S , a protein that has been shown to be specifically required for transcription of a series of genes required for successful entry into stationary phase. In normal transition to stationary phase, selective association of σ^S with RNA polymerase leads to the expression of a large regulon of genes that cumulatively cause *E. coli* to convert their shape from a rodlike form to a smaller, more spherical form, and to become generally stress-resistant (Hengge-Aronis, 1993). In *rpoS*⁻ mutant cells, there are general defects in the response to cell stress and reduced survival of stationary phase; strikingly, because of misregulation of the *bolA* gene, cell morphology is significantly affected, with *rpoS*⁻ cells generally larger than wild-type cells, and displaying a series of morphologies ranging from long filaments to short rods (Lange and Hengge-Aronis, 1991). The presence of the RPB4/RPB7 subcomplex in pol II has been demonstrated to alter polymerase initiation

properties (Edwards *et al.*, 1991); these properties may also include promoter selectivity, as some data on transcription patterns in *rpb4*⁻ yeast would suggest (Choder, 1993; Choder and Young, 1993). By the *rpoS* model, the development of excessive cell elongation in the presence of excess hsRPB7 and RPB7 is compatible with the idea that RPB7 may function as a stressor-specific σ^S -like factor in lower and potentially higher eukaryotes, affecting a range of cell functions including control of cell morphology. The association of RPB7 with *rpoS*-like functions is consistent with current evidence that suggests that sigma-like functions are dispersed among several proteins in eukaryotes (Jaehning, 1991).

Finally, a striking and unexpected finding of this study is the degree of complexity associated with hsRPB7 expression at the RNA and protein levels. The relative abundance of the hsRPB7 transcript varies drastically relative to that of the mRNA encoding the largest RNA pol II subunit in different tissues. Antibody to hsRPB7 detects multiple cross-reacting proteins that are expressed at variable levels in different human cell lines. Because the RNA analysis was done with primary tissue, and the protein analysis with cultured cell lines, it is not possible to directly compare expression levels based on these data. For at least some tissues, the RNA and protein data show parallel trends, putting hsRPB7 expression higher in kidney tissue and kidney-derived 293 cells, and lower in pancreatic tissue and pancreas-derived PANC-1 cells. Subsequent analysis should clarify both the magnitude of variation of hsRPB7 protein expression, and the significance of this variation for biological function *in vivo*. A particularly intriguing question arising from the protein expression data is the role of the 24-kDa form of hsRPB7. One possibility is that this species corresponds to an inactive pool of hsRPB7 held in reserve; alternatively, this species may possess an entirely discrete function. In sum, our results raise the possibility that differing forms of RNA polymerase II, some with and some without associated hsRPB7, exist in different tissues, paralleling the existence of pools of pol II with and without the RPB4/RPB7 subcomplex noted in *S. cerevisiae* (Choder and Young, 1993). If so, this might lend yet another twist to the complex field of higher eukaryotic transcriptional regulation.

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